Evolution of Moth Sex Pheromone Desaturases

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Moth sex pheromone communication has evolved to use complex blends of relatively simple long-chain fatty acid precursors. Species specificity is derived from the unique stereochemistry of double bonds introduced into exact locations along the hydrocarbon backbone of fatty acids, which are reduced and then undergo a variety of chain-shortening and functionalization reactions to form the pheromone blend. Key enzymes that have evolved to function in this system are the acyl coenzyme A desaturases, which catalyze the introduction of the double bonds. This report gives an overview of the evolution of these enzymes, with an introduction to the newly arisen field of "semiochemical genetics."

Key words: **pheromone; desaturase; speciation; semiochemical genetics**

Introduction

Lepidoptera are among the most diverse groups of animals, numbering more than 110,000 named species worldwide. Many of these species are serious agricultural pests, such as the European corn borer, which is responsible for an estimated $1 billion reduction in corn yield annually in the United States.[1] A substantial amount of research effort has been expended on means to control moth pests to mitigate this problem. A major effort focuses on moth sex pheromone biology.

To attract a male, a female moth emits a blend of volatile, long-chain fatty acid hydrocarbons that act as a pheromone.[2] The pheromone can travel long distances (several kilometers in some species) and is precisely constructed to attract a male of the correct species. The molecules making up this pheromone blend are long-chain fatty acid hydrocarbons that are relatively simple in structure. Over the course of moth evolutionary history, a system has evolved in which species specificity is ensured through the use of complex blends of these simple hydrocarbons. To synthesize the complex blends, three key enzymatic reactions play a role: (1) chain shortening of the hydrocarbon backbone, (2) reduction of acyl intermediates to alcohols, and (3) desaturation at specific locations along the hydrocarbon chain.

The enzymes that mediate the desaturation of long-chain fatty acids used to form moth sex pheromone blends have been the subject of much study for nearly three decades. The enzymes have been identified as acyl coenzyme A (acyl-CoA) desaturases and form a multigene family.[3,4] Only within the past few years have studies been conducted on the molecular evolution of these genes. These studies have revealed several different enzymes whose functional relationships are mirrored in their phylogenetic clustering patterns.[5] However, several genes exist whose function has yet to be determined, including a class of recently discovered desaturase genes that have fused with a retroposon.[6]

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This report reviews the evolution of moth sex pheromone desaturases.

System of Nomenclature

One can separate the acyl-CoA desaturases of moths into several different functional classes on the basis of their activity and substrate specificity. For example, an enzyme that catalyzes the introduction of a double bond at the ninth carbon atom of the hydrocarbon chain is known as a Δ9 desaturase. If the enzyme acts on molecules that contain either 16 or 18 carbon atoms but shows a preference for the former, it is termed a Δ9(16>18). If the former were favored as a substrate, it would be termed a Δ9(18>16) desaturase. Also, certain enzymes are capable of multiple desaturase activities and are named accordingly, such as the Δ10–12 desaturases of *Bombyx mori* and *Spodoptera littoralis* that catalyze the introduction of double bonds at the 10th, 11th, or 12th carbon atoms.[7,8]

To date, several different functional classes have been characterized. They include the Δ9(16>18), Δ9(18>16), Δ9(14–26), Δ10, Δ11, Δ12, and Δ14 enzymes from a variety of species; the multifunctional Δ10–12 desaturases just mentioned; and an unusual multifunctional enzyme from *Thaumetopoea pityocampa* that possesses Δ11 and Δ13 desaturase activities as well as Δ11 acetylenase activity.[5,9,10]

Knipple *et al.* proposed an alternative system of nomenclature based on phylogenetic clustering patterns.[11] According to this system, certain amino acid residues that are conserved among all members of a phylogenetic clade of acyl-CoA desaturases are used to name the enzymes that belong to that clade. A species abbreviation is also incorporated into the name for each enzyme. For instance, HassKPSE is an enzyme from *Helicoverpa assulta* that possesses the conserved amino acid motif KPSE.[12] In this report, I do not use this alternative classification scheme because it does not convey functional information, which is critical to understanding the evolution of acyl-CoA desaturases.

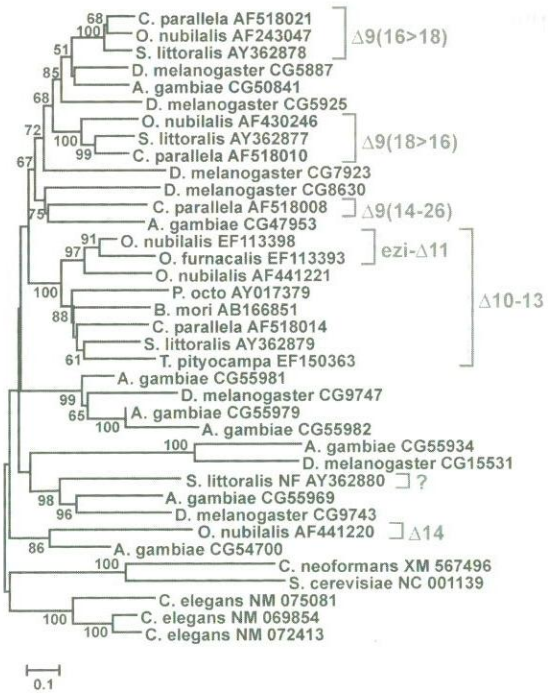

Figure 1. Phylogeny of representative acyl-CoA desaturases from moths and dipterans. Moth sex pheromone desaturases are bracketed in red. The tree was constructed from Poisson amino acid distances by using the neighbor-joining method with 1000 bootstrap pseudoreplicates to assess clade support; the program MEGA4 was used to conduct the analysis.[17] Desaturase protein sequences from *Caenorhabditis elegans* and two fungi (*Saccharomyces cerevisiae* and *Cryptococcus neoformans*) were included as outgroup taxa. The genes of *D. melanogaster* and *A. gambiae* were extracted from their genome databases and denoted by their gene identification numbers. The GenBank accession numbers for all other genes are given after their species names. (In color in *Annals* online.)

Phylogenetic Relationships

Several studies have examined the phylogenetic affiliations of moth sex pheromone desaturase enzymes.[4–6,9,11,13] To summarize partial or missing information in some of these previous studies, Figure 1 gives a phylogeny of the major functional classes of moth sex

pheromone desaturase enzymes and related sequences from the *Drosophila melanogaster* and *Anopheles gambiae* dipteran genomes.

One can glean two important pieces of information from the phylogeny in Figure 1. First, moth sex pheromone desaturases do not form a monophyletic group. Instead, certain functional classes are more closely related to dipteran genes than they are to other moth genes. This finding indicates that the acyl-CoA desaturase multigene family evolved before moths and flies diverged.[4,9] Second, the clustering pattern indicates that acyl-CoA desaturases must have been recruited to function in sex pheromone communication independently, multiple times. Otherwise, if the use of acyl-CoA desaturases for sex pheromone biosynthesis had arisen only once in moths, all such desaturases would form one monophyletic group, which they do not.

In that context, the *D. melanogaster desat*1 and *desat*2 genes (denoted in the published genome database as CG5882 and CG5925, respectively) are part of a clade that includes the moth Δ9(16>18) genes and a putative desaturase from the mosquito, *A. gambiae*, that still awaits functional characterization. The gene *desat*1 functions in the sex pheromone biosynthetic pathway for *D. melanogaster* via the biosynthesis of cuticular diene hydrocarbons, and *desat*2 also has been implicated to play a role in that regard in at least some strains of *D. melanogaster*.[14,15] The clustering of the *desat*1 and *desat*2 genes with the moth Δ9(16>18) genes suggests that desaturases were co-opted for sex pheromone biosynthesis before the divergence of dipterans and lepidopterans. In fact, some similarity exists between the pheromones of trichopterans and those of lepidopterans, suggesting that they should also be present in that order.[16] However, the genes might have been independently co-opted after all three orders diverged. Answering this question requires a phylogenetic study that includes several primitive lepidopteran, dipteran, and trichopteran taxa, along with functional characterization of the included desaturase genes.

Desaturase Homologues with Unknown Functions

Several genes have been identified that are clearly homologous to sex pheromone desaturases yet fail to yield a desaturated gene product when assayed for function. Such genes have been referred to as nonfunctional (NF) with respect to pheromone biosynthesis. One such gene from *S. littoralis* was included in the phylogenetic analysis conducted for this article. As shown in Figure 1, this gene is highly divergent from the main Δ9 and Δ10–13 sex pheromone desaturase groups and clusters with a gene from *D. melanogaster* and a gene from *A. gambiae*. A high bootstrap value (98%) supports the close relationship of these three genes and suggests that all three are orthologues that may share the same function. Serra *et al.* urged studies to begin assaying for functions, such as acetylenase and hydroxylase activity, to determine whether desaturases are multifunctional.[10] The same should be done for the NF genes that do not function as desaturases but that may function as some other type of enzyme.

Perhaps the NF genes of moths could evolve desaturase activity *de novo*. Like the *S. littoralis* NF gene, the Δ14 gene of *Ostrinia* species is highly divergent from the main Δ9 and Δ10–13 desaturase groups and clusters more closely with fruit fly and mosquito genes than with other moth genes. To speculate that it evolved desaturase function recently is tempting because no other lineage of moth is known to possess a functional Δ14 gene.[9] Interestingly, Xue *et al.* identified a previously unknown group of desaturase homologues sequences present in the genomes of *O. furnacalis* and *O. nubilalis*.[6] The genes apparently formed from a fusion between a Δ11 gene and a long interspersed element, which is a type of retroposon. What function, if any, these genes possess is unknown. However, that the genes retain intact open reading frames after at least 1 million years of divergence time between the two species of *Ostrinia* in which they were detected suggests that they may be functional.[6]

A Role in Speciation

Roelofs *et al.* showed that changes in desaturase genes can induce speciation in moths.[9] These authors studied *O. nubilalis* and *O. furnacalis* and showed that the form used a Δ11 desaturase to synthesize its female sex pheromone, whereas the latter used a Δ14. Presumably, this shift was brought about through a switch to the use a Δ14 gene in *O. furnacalis* that had evolved to function as a desaturase. As soon as this happened and the enzyme was recruited to function in the pheromone gland, the sex pheromone blend of the ancestral *O. furnacalis* population changed, thereby isolating it from other (presumably Δ11 utilizing) populations, which either went extinct or diverged into other species. This phenomenon can be termed "pheromone shifting" and involves gene duplication, gene loss, and/or nonfunctionalization or silencing.[4]

Several genetic mechanisms can bring about pheromone shifting. The most obvious involves a gene duplication event followed by functional divergence of the duplicate. If the duplicate "drops out" of the sex pheromone biosynthesis pathway, it will have no further effect on sex pheromone biosynthesis and, by extension, will not be involved in pheromone shifting. However, if it remains in the pathway, the blend can change through the addition of new products made by the functionally divergent duplicate. If the original gene drops out of the pathway, then a different pheromone blend can result.

However, recent gene duplication need not happen for pheromone shifting to occur if several paralogous gene sequences exist that are close enough in function to be co-opted into the sex pheromone biosynthesis pathway. Presumably, this scenario explains the evolution of the new Δ14-synthesized blend of *O. furnacalis*. Molecular clock studies reveal that moth desaturase genes emerged long before lepidopterans and dipterans diverged.[4,9] This finding explains why, for example, the moth Δ14 gene clusters with dipteran genes instead of other moth genes (Fig. 1). It also indicates that the Δ14 gene was already present in the genome of the *O. furnacalis* ancestor before it was recruited into the sex pheromone biosynthetic pathway. Also, the desaturase function must have evolved independently in the Δ14 gene before its recruitment into the pathway, because the gene is not involved in the sex pheromone biosynthetic pathways in closely related species.[9] This scenario may also explain the evolution of the Δ9(14–26) gene in *Choristoneura parallela*.[5] In theory, gene loss can drive pheromone shifting if either multiple desaturases take part in the pathway or a paralogue can be recruited into the pathway in the event that only one desaturase is involved. Evidence for this process, however, has yet to be found.

Conclusions

The sex pheromone desaturase genes of moths offer a variety of interesting questions to pursue in genomics, genetics, biochemistry, chemical ecology, and molecular evolutionary biology. In fact, the knowledge and techniques from all these fields have already been applied to several studies on moth sex pheromone desaturases.[5,9–11,13] This newly emerging integrative field that combines molecular, biochemical, and ecological sciences can be termed "semiochemical genetics." Seeing whether it becomes the preferred approach for future studies of pheromone biology will be interesting.

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Conflicts of Interest

The author declares no conflicts of interest.

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